

Scotland's Rural College

Behavioural adaptation to a short or no dry period with associated management in dairy cows

Kok, A; van Hoeij, RJ; Tolkamp, BJ; Haskell, MJ; van Kneegsel, ATM; de Boer, IJM; Bokkers, EAM

Published in:
Applied Animal Behaviour Science

DOI:
[10.1016/j.applanim.2016.10.017](https://doi.org/10.1016/j.applanim.2016.10.017)

First published: 07/11/2016

Document Version
Peer reviewed version

[Link to publication](#)

Citation for pulished version (APA):

Kok, A., van Hoeij, R.J., Tolkamp, B.J., Haskell, M.J., van Kneegsel, A.T.M., de Boer, I.J.M., & Bokkers, E.A.M. (2016). Behavioural adaptation to a short or no dry period with associated management in dairy cows. *Applied Animal Behaviour Science*, 186, 7 - 15. <https://doi.org/10.1016/j.applanim.2016.10.017>

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

- Users may download and print one copy of any publication from the public portal for the purpose of private study or research.
- You may not further distribute the material or use it for any profit-making activity or commercial gain
- You may freely distribute the URL identifying the publication in the public portal ?

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Behavioural adaptation to a short or no dry period and associated management in dairy cows

Akke Kok^{a,b,*}, Renny J. van Hoeij^b, Bert J. Tolkamp^c, Marie J. Haskell^c, Ariëtte T.M. van Knegsel^b, Imke J.M. de Boer^a, Eddie A.M. Bokkers^a

^aAnimal Production Systems group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands

^bAdaptation Physiology group, Wageningen University, P.O. Box 338, 6700 AH Wageningen, the Netherlands

^cScotland's Rural College (SRUC), West Mains Road, Edinburgh EH9 3JG, United Kingdom

*Corresponding author: akke.kok@wur.nl +31 317484626

ABSTRACT

From calving, dairy cows are typically milked for about a year, and subsequently managed to have a non-lactating or 'dry period' (DP) before next calving. However, the DP may reduce cow welfare because of many DP-related changes in management and a severe negative energy balance after calving. Shortening or omitting the DP may have beneficial effects on cow welfare through fewer changes in management before calving, and a lower milk yield after calving. Our objective was to assess the effects of a short DP (30 days) and no DP on feeding, lying, and number of steps of dairy cows in late gestation and early lactation. Feeding behaviour was recorded by computerized feeders for 122 periods (42 with a short DP and 80 with no DP) from week -6 to week 7 relative to calving. Steps and lying behaviour of 81 of these cows (28 with a short DP and 53 with no DP) were recorded with accelerometers in week -4 and in week 4 relative to calving only. Effects of DP treatment and parity on behaviour were analysed with mixed models. Before calving, cows with a short DP were fed a DP ration, and moved to a dry cow group. During this time, cows with a short DP spent more time lying (13.7 vs. 12.6 h per day; $P = 0.01$) and feeding (240 vs. 209 min per day; $P < 0.01$), and stepped less (663 vs. 1130 steps per day; $P < 0.01$) than cows with no DP. After calving, all cows were fed the same lactation ration and were housed in the same herd. Cows with a short DP, however, had a lower feed intake (35.7 vs 39.1 kg per day; $P < 0.01$), and spent less time lying (10.7 vs. 11.6 h per day; $P = 0.03$) after calving than cows with no DP. Milk yield was negatively correlated with daily lying time ($r: -0.22$; $P < 0.05$), but was not correlated with daily feeding time. Also, less time was spent on both lying and feeding after calving than before calving. These results indicate that lying time was not constrained by feeding time. Lying time was positively correlated with energy balance ($r: 0.28$; $P < 0.01$). No DP, in comparison with a short DP and the associated changes in management, reduced lying time and increased the number of steps in late gestation, and resulted in a higher feed intake and longer lying time in early lactation.

Keywords: rest period, lying, feeding, cattle, transition period, sensor data

1. INTRODUCTION

The lactation cycle of dairy cows starts with calving. From calving, cows are typically milked for about a year, and subsequently managed to have a non-lactating or 'dry period' (DP) of 6 to 8 weeks before next calving. The DP allows for treatment of intramammary infections (Robert et al., 2006), facilitates the renewal of udder cells (Capuco et al., 1997), and maximises milk yield in the next lactation (Kuhn et al., 2005; van Knegsel et al., 2013). The DP is generally considered a rest period for the cow that allows for reduced metabolic and physical activity in the last two months of pregnancy.

Whether a DP is beneficial for dairy cow welfare has been questioned (Zobel et al., 2015). Good welfare has been defined as feeling well, functioning well, and living a natural life (Fraser et al., 1997). Planned cessation of lactation, as well as being unnatural, was shown to increase udder pressure and stress (as measured by faecal glucocorticoid metabolites) at the start of the DP (Tucker et al., 2009; Bertulat et al., 2013). In addition, cows need to adapt to a new social environment and to dietary changes at the start and end of the DP, because they are typically moved to a non-lactating group and fed a dry cow diet (von Keyserlingk et al., 2008; Martens et al., 2012; Santschi and Lefebvre, 2014). After the DP, a higher milk yield is associated with a more severe negative energy balance (Rastani et al., 2005; van Knegsel et al., 2014). Such a negative energy balance is associated with impaired fertility and reduced metabolic health (Butler, 2003; Chen et al., 2015a; b), and may last until 3 months into lactation after a conventional DP (Rastani et al., 2005; van Knegsel et al., 2014). As a consequence of the prolonged lipolysis to meet energy needs, and possibly conflicting motivations such as hunger, inappetence, and the desire to rest or ruminate, a cow may be in a negative affective state during this period (Roche et al., 2009).

Behavioural adaptation may not interfere with welfare as long as it is within the limits of the adaptive capacity of the animal (Korte et al., 2007). Behaviour of cows is affected by external factors (such as housing) and internal factors (such as behavioural needs). The behaviour patterns that are expressed are the result of these internal and external factors. Behaviour patterns can be assessed by examining

the time budget and the temporal distribution of behaviours (Winter and Hillerton, 1995). Much of the time budget of dairy cattle is made up of lying, feeding, ruminating, and – in lactating cows – being milked (Gomez and Cook, 2010; Norring et al., 2012). The daily duration of these activities depends on factors such as housing, access to pasture, milking facilities, lameness, and stage of lactation (Krohn et al., 1992; Huzzey et al., 2006; Fregonesi et al., 2007; Gomez and Cook, 2010). In addition to changes in feeding time, cows were found to increase feeding rate when given limited access to resources (Munksgaard et al., 2005) and when lame (González et al., 2008). To understand such changes in short-term feeding behaviour, it is informative to cluster visits to the feeder into distinct feeding bouts (meals) (Yeates et al., 2001; Tolkamp et al., 2002). Cow welfare may be compromised when cows cannot adapt their behaviour to the circumstances, or if short-term behaviour patterns result in a long-term reduction of welfare. Increased standing time, for example, is observed in early lactation (Fregonesi and Leaver, 2001; Munksgaard et al., 2005), but (on hard surfaces) is a risk factor for lameness (Cook and Nordlund, 2009).

Shortening or omitting the DP may have beneficial effects on cow welfare (Zobel et al., 2015). Both strategies improve the energy balance after calving, through a reduced milk yield and equal or better feed intake after calving (Rastani et al., 2005; van Knegsel et al., 2014). Moreover, milk yield before dry-off is lower for a short DP than for a standard DP (Pezeshki et al., 2007), because milk yield decreases towards the end of lactation. A lower milk yield before dry-off reduces udder pressure and stress in the DP (Bertulat et al., 2013), and reduces the risk of intramammary infections after calving (Rajala-Schultz et al., 2005). Cows with no DP can be kept in the herd, without regrouping and dietary changes.

It is unclear how dairy cows adapt behaviourally to a DP, and how the absence of a DP affects their time budget. Our objective, therefore, was to assess the effects of a short DP and no DP and associated management on feeding, lying, and number of steps of dairy cows in late gestation and early lactation. To assess possible reasons for changes in behaviour, we also studied associations between behaviour, milk yield, and energy balance in early lactation.

2. MATERIAL AND METHODS

2.1 Experimental design, animals, and housing

The Institutional Animal Care and Use Committee of Wageningen University approved the experimental protocol in compliance with Dutch law on Animal Experimentation (protocol number 2014125). The experiment was conducted at the Dairy Campus research farm (Lelystad, the Netherlands) using 125 Holstein-Friesian cows between January 2014 and July 2015. The study was initially designed to analyse the effect of DP length and dietary energy source on energy balance and metabolic health; sample size was based on a power analysis for these variables. Cows were included in the experiment at an average rate of 3 cows per week, based on the availability of cows in late gestation. Inclusion criteria were an expected calving interval shorter than 490 days, a milk yield of >16 kg and no clinical or subclinical mastitis (a cell count > 250.000 cells/ml) at 90 days before expected calving. For practical reasons, six cows were used twice in the experiment, resulting in data for 131 periods around calving (60 periods of cows in parity 1 before calving and 71 periods of cows in parity > 1 before calving).

Treatment groups were balanced for parity (1 or > 1 before calving), expected calving date, and milk production in the previous lactation. This was done by distributing clusters of 6 similar cows randomly over no DP (n=87), or a short DP of 30 days (n=44), using a random number generator. Twice as many cows were assigned to the no DP treatment because of an additional contrast in concentrate allowance (further details will be given below).

Cows entered the experiment on Mondays, 44 ± 3 days before the expected calving date and were kept in the study until 305 days in milk. All cows were housed in the same freestall barn with a concrete slatted floor, and stalls fitted with rubber mattresses covered with sawdust. Lactating and dry cows were kept in separate groups. The stocking density in both groups was maintained at one cow per cubicle and two cows per feeding bin throughout the experiment, with a space allowance of 7 m² per cow.

The drying-off protocol for cows with a short DP consisted of an abrupt transition to the DP ration at day 7 before dry-off and an abrupt transition to milking once daily at day 4 before dry-off. Cows were dried off (i.e. milked for the last time) on Mondays on 30 ± 3 days before the expected calving date.

At dry-off no antibiotics were used. Dry cows were weighed in the milking parlour on Tuesdays.

Lactating cows were milked and weighed in the milking parlour twice daily at about 06.00 h and 17.00 h.

2.2 Feed composition and provision

During the DP, cows received a DP ration (estimated net energy (NE): 5.4 MJ per kg DM) that consisted of grass silage, maize silage, wheat straw, and rapeseed meal in a ratio of 48:19:25:8 (DM basis), and vitamins and minerals (for more detail see Van Hoeij et al., under review). Cows with no DP received a lactation ration (estimated NE: 6.4 MJ per kg DM) that consisted of grass silage, maize silage, wheat straw, soybean meal, and sugar beet pulp in a ratio of 45:35:2:8:10 (DM basis), and vitamins and minerals. After calving, all cows received this lactation ration up to 49 days in milk (DIM).

Basal rations were provided in roughage intake control (RIC) feeders (Insentec, Marknesse, the Netherlands). One RIC feeder was available per two cows. Rations were mixed once daily before 10.00 h and fed twice daily around 10.00 h and 17.00 h. The RIC feeders could not be accessed by the cows when feeders were filled and from 23.45 h to 0.00 h when data records were saved. Cows had free access to water, that was provided in valve trough drinkers placed in between feeding bins and quick drainage troughs of 150L at opposite sides of the barn. Because cow density was kept constant, lactating cows had access to 3 or 4 troughs and dry cows had access to 1 or 2 troughs depending on group size.

Concentrate was provided separately from the basal ration, and the concentrate allowance differed between treatment groups. Cows with a short DP were fed a standard amount of concentrate for their expected milk yield (Short DP STD). The expected daily milk yield in the 14 weeks after calving was 40.4 kg fat-and-protein-corrected milk (FPCM) for cows with a short DP and 35.4 kg FPCM for cows with no DP, compared with 43.3 kg FPCM for cows with a standard DP (Van Knegsel et al., 2014).

Cows with no DP were assigned either to the same concentrate level as cows with a short DP (No DP STD), or to a lower concentrate level that matched their expected milk yield (No DP LOW), .

All cows received concentrate (869 g DM per kg; estimated NE: 7.4 MJ per kg DM) at a level of 1 kg per day from -10 ± 3 days before the expected calving date. The concentrate allowance increased

stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up to 8.5 kg per day at 28 DIM for the short DP STD and no DP STD treatments, and stepwise by 0.3 kg per day from 1.0 kg per day at 4 DIM up to 6.7 kg per day at 22 DIM for the no DP LOW treatment. Concentrate was provided by two computerized feeders located in the freestall (Manus VC5, DeLaval, Steenwijk, the Netherlands). The individual daily allowance of concentrate was available in equal portions (minimum portion size: 0.4 kg) over six 4-h periods, and the actual quantity dispensed (kg per day) was recorded. Uncollected concentrate portions in one timeslot were added to the portion in the next timeslot. Additionally, lactating cows received 0.5 kg of a standard concentrate (887 g DM per kg; estimated NE: 7.7 MJ per kg DM) when they were milked (i.e. 1.0 kg per day).

2.3 Measurements and data analysis

2.3.1 Feeding behaviour

For each visit to a feeder, RIC feeders recorded cow identity, the start time and end time (hh:mm:ss) of the visit, and the start weight and end weight of the feed in the feeder to the nearest 0.1 kg. Visit duration (s), feed intake (kg), and feeding rate (kg per min feeding) were calculated from these records. Concentrate feeders only registered the amount of concentrate collected per cow per day. RIC data were analysed from 6 weeks before calving until 7 weeks after calving. In total, 9 cows were excluded from the analysis for various reasons: 5 cows were removed from the experiment before 7 weeks in lactation for health reasons (severe clinical lameness (2x), broken hip, 2 deaths within 10 days after calving), and 4 cows did not have the assigned DP length due to early calving in case of the short DP group (n=1) and spontaneous drying off (i.e. the cow stopped lactating despite twice-daily milking; n=3) in case of the no DP group. The RIC dataset consisted, therefore, of 122 13-week periods, with a total of 332,524 recorded visits to the RIC feeders.

Criteria were used to clean the dataset prior to analysis. Visits with a feeding rate > 2 kg per min were discarded (0.4% of records), because inspection of sequentially recorded visits to the same feeder suggested that these records were erroneous. In addition, visit duration was discarded for visits that lasted longer than 3 h and visits with feeding rates below 0.02 kg per min (0.1% of records). Inspection of these records suggested that the recorded feed intakes were genuine for these visits, as

evidenced by sequentially recorded feeding bin weights, whereas visit durations were likely long because of failed registration of the end time of the visit.

Visits were clustered based on the interval length between visits. For dairy cows, the distribution of short intervals within meals and longer intervals between meals can be described by a three-population model, which uses a combination of two Gaussian distributions for the short intervals and one Weibull distribution for the longer intervals (for further details see: Yeates et al., 2001). A meal criterion can be estimated from this distribution, to classify intervals as within meal and between meal intervals in the most accurate way. When the interval between visits is shorter than the meal criterion, the visits belong to the same meal.

Visit records were used to compute intervals between subsequent visits for each cow. A three-population model was fitted to the frequency distribution of the \log_e -transformed intervals between visits, and a meal criterion was estimated from this distribution (Yeates et al., 2001; Tolkamp et al., 2002, 2011). To assess whether separate meal criteria for treatment groups or periods relative to calving would be more appropriate than one single meal criterion for all treatments, nested models were constructed. Three nested models were produced to estimate separate meal criteria for 1) the three treatment groups (Short DP STD, No DP STD, and No DP LOW), 2) the two periods (before and after calving), and 3) each treatment \times period interaction. Comparisons of the log-likelihoods of nested models using likelihood ratio tests showed that the separate factors and their interaction all improved model fit. However, the resulting meal criteria were very similar between treatment groups before calving (18.1, 17.4, and 17.7 min) and after calving (21.9, 20.2, and 21.2 min). Therefore, it was decided to use one meal criterion before calving (18.0 min) and one meal criterion after calving (20.9 min), calculated from the pooled data. These meal criteria were used to cluster visits into meals.

Duration of meals (meal duration), duration of visits within meals (feeding duration), number of visits per meal, and feed intake per meal were calculated, and secondary variables (e.g. daily feed intake, feed duration and feeding rate) were derived from these variables. Weekly means of feeding behaviour characteristics per cow per day were used for the analysis.

Mixed models were used to analyse the effect of fixed factors treatment, parity (1 or > 1 before calving), and week, as well as interactions of parity and week with treatment, on feeding behaviour

(PROC MIXED procedure in SAS version 9.1; SAS Institute Inc., Cary, NC). The combination of cow identity and parity before calving was specified as repeated subject. No DP STD and No DP LOW were grouped together (No DP), because preliminary analysis showed no difference for this. The covariance structure with the best model fit, based on the lowest Akaike's information criterion, was selected from unstructured, compound symmetry, and autoregressive covariance structures. Statistical significance ($P < 0.05$) of fixed effects was evaluated with approximate F tests (Kenward and Roger, 1997); treatment contrasts were compared using Wald tests.

2.3.2 Lying behaviour and steps

Lying behaviour and steps were recorded with triaxial accelerometers (IceQube, IceRobotics, South Queensferry, UK) from June 2014 until July 2015. Lying behaviour is recorded when the hind leg is in a horizontal position; the step count measures the number of times the animal lifts its leg up and places it back down again. The step count was used as indicator for walking activity, although stepping may also be recorded while standing in one place (e.g. during milking). Sensors were attached to the left or right hind leg and detached on Thursdays between 10.00 h and 12.00 h. Each cow was herded into a cubicle for the attachment of the sensor. Because of limited sensor availability, lying behaviour and steps of cows were recorded for 6 complete days (Friday until Wednesday) at 4 weeks (26 ± 3 to 21 ± 3 days) before the expected calving date, and at 4 weeks (22 ± 3 to 27 ± 3 days) after calving only. Cows were regrouped and switched to a DP ration 11 days before the precalving measurement period, and dried off 4 days before the precalving measurement period. We therefore expect to measure little short-term behavioural responses to the change in diet, change in social environment, or the process of drying off (von Keyserlingk et al., 2008; Tucker et al., 2009). Lying behaviour and steps of 81 unique cows were recorded in both periods ($n=26$ for no DP STD; $n= 27$ for no DP LOW; and $n= 28$ for short DP STD), including only cows that were also included in the analysis of feeding behaviour.

Data were downloaded from IceQube sensors using IceReader, and processed by IceManager (both from IceRobotics, South Queensferry, UK) to produce two data files per cow per time period. One file contained all recorded lying bouts, with a start date, start time (hh:mm:ss) and duration (s); the other file consisted of recorded lying time (s), standing time (s), and number of steps per 15-min interval.

Recorded lying bouts with durations shorter than 33 s were discarded as false lying bouts (Kok et al., 2015). The filtered data of lying bouts were used to compute the number of lying bouts per cow per day. Daily lying time and number of steps were computed from the 15-min summaries. Weekly means of lying bouts, lying time and steps per cow per day were used for the analysis.

To analyse the effect of treatment (no DP or short DP), parity (1 or >1 before calving), and week on lying time, number of lying bouts, and steps, the same mixed model approach was used as for the analysis of feeding behaviour characteristics. No DP STD and No DP LOW were grouped together (No DP), because preliminary analysis showed no difference for this.

The daily number of steps in the period before calving was compared between days of the week, to assess the impact of going through the milking parlour for weighing on Tuesdays for cows with a short DP. Per treatment, the mixed model included a fixed effect for day of the week (Friday through Wednesday), and a random cow effect. All weekdays were compared using pairwise Wald tests with Tukey-adjusted P-values, and the estimate statement was used to compare the number of steps recorded on Tuesdays with all other days.

2.3.3 Associations between behaviour, milk yield, and energy balance

To assess possible reasons for differences in behaviour, we analysed associations between behaviour, milk yield and energy balance at 4 weeks after calving. Milk yield was recorded daily. Energy balance was calculated according to the Dutch net energy for lactation (VEM) system (Van Es, 1975) as the difference between intake of VEM with the requirement of VEM for maintenance, milk production, and pregnancy (1,000 VEM = 6.9 MJ of NE). Energy balance was expressed in $\text{kJ per kg}^{0.75}$ per day (Van Es, 1975). Computation of the energy balance required milk yield, milk composition, body weight of the cow, feed intake, and energy content of the feed. Milk samples for fat and protein analysis (ISO 9622, Qlip, Zutphen, the Netherlands) were collected for four subsequent milkings and were analysed as a weighted pooled sample per cow. RIC feeders recorded feed intake of the basal ration (kg) and concentrate feeders recorded the quantity of concentrates dispensed (kg) per cow per d. Feed intake was converted to energy intake using the dry matter content and net energy (NE) of each diet component.

Means and standard deviations of milk yield and energy balance in week 4 after calving were

computed per DP treatment (no DP or short DP) per parity (2 or >2) . Associations between variables were assessed with Pearson correlations. Significant correlations (r ; $P < 0.05$) were interpreted as slight (< 0.2), low (0.2 - 0.4), moderate (0.4 - 0.7), high (0.7 - 0.9), or very high (> 0.9) (Martin and Bateson, 1993).

3. RESULTS

3.1 The effect of a short or no DP on feeding behaviour

Over the 6 weeks before calving, cows with a short DP and cows with no DP had on average 7 meals per day with 5 visits per meal from the RIC feeders (i.e. excluding concentrate; Table 1). Average meal duration (i.e. the time from the start of the first visit within the meal until the end of the last visit within the meal), however, was longer for cows with a short DP than for cows with no DP, which resulted in total meal times of 293 min per day for cows with a short DP and 255 min per day for cows with no DP. The feeding duration (i.e. the time spent with head in the feeder) was about 80% of the meal duration for both treatments. Meal size (i.e. the feed intake per meal) and feed intake per day were smaller for cows with a short DP than for cows with no DP, and cows with a short DP had a lower feeding rate. Young cows (parity 1) had longer total feeding times and lower feeding rates than older cows (parity >1) (Figure 1c, 1d).

Over the 7 weeks after calving, cows with a short DP and cows with no DP had on average 8 meals per day with 4 visits per meal from the RIC feeders (Table 2). The number of meals per day and feeding rate peaked in the first week after calving, whereas total feeding time and feed intake were lowest in this week (Figure 1). Average meal duration and total meal time were not different between DP treatments after calving. Meal size was not different between cows with a short DP and cows with no DP, but feed intake per day was 3.4 kg (1.3 kg DM) per day lower for cows with a short DP than for cows with no DP. Young cows (parity 1 before calving) with a short DP had longer total feeding times ($P = 0.04$) and lower feeding rates ($P < 0.01$) than young cows with no DP (Figure 1c, 1d).

Looking at the diurnal pattern of feeding, cows spent more time in a meal during daytime than during the night, with the highest proportion of cows having meals after fresh feed delivery, peaking around noon (Figure 2). Before calving, cows with a short DP spent more time in a meal during daytime than cows with no DP (Figure 2a). After calving, the diurnal pattern of meals was similar for cows with a short DP and cows with no DP (Figure 2b).

3.2 The effect of a short or no DP on lying behaviour and steps

The number of lying bouts per day was not affected by DP treatment or period relative to calving (Table 3). Young cows (parity 1 before calving) had 13.2 (SE: 0.5) lying bouts per day, whereas older cows (parity > 1) had 11.4 (SE: 0.5) lying bouts per day ($P < 0.01$).

Daily lying time was affected by a DP treatment \times period interaction. At 4 weeks before calving, daily lying time was 1.1 h longer for cows with a short DP than for cows with no DP ($P = 0.01$). At 4 weeks after calving, however, daily lying time was 0.9 h shorter for cows with a short DP than for cows with no DP ($P = 0.03$). The change in lying time between the period before calving and the period after calving was more extreme for cows with a short DP (-3 h) than for cows with no DP (-1 h).

Before calving, time spent lying dipped during milking for cows with no DP, whereas this was not the case for cows with a short DP (Figure 3a). After calving, lying patterns were similar for cows with a short DP and cows with no DP (Figure 3b).

The number of steps per day was affected by a DP treatment \times period interaction (Table 3). Before calving, cows with a short DP had 41% lower step counts than cows with no DP. After calving, the number of steps did not differ between DP treatments, and was similar to the number of steps of cows with no DP during the period before calving.

Before calving, cows with a short DP were weighed in the milking parlour on Tuesdays. To assess the impact of this additional exercise on daily number of steps, step counts were compared between days of the week. On average, 220 (SE: 29) more steps were recorded for cows with a short DP on Tuesdays than on Wednesdays through Mondays ($P < 0.01$; Figure 4). Cows with a short DP also had

lower step counts during weekends than on weekdays. Cows with no DP had no increased step count on Tuesdays during the period before calving. Their step count was highest on Mondays, which was the day animals were regrouped (although focal cows were not moved in this period).

3.3 Associations between behaviour, milk yield, and energy balance.

Mean milk yield of cows with a short DP was 37.9 kg (SD: 6.0) per day for cows in parity 2 and 36.8 kg (SD: 6.3) per day for older cows; mean milk yield of cows with no DP was 29.5 kg (SD: 5) per day for cows in parity 2 and 35.3 kg (SD: 8.1) per day for older cows. Mean energy balance of cows with a short DP was -191 kJ per kg^{0.75} (SD: 150) per day for cows in parity 2 and -179 kJ per kg^{0.75} (SD: 190) per day for older cows; mean energy balance of cows with no DP was 44 kJ per kg^{0.75} (SD: 113) per day for cows in parity 2 and -94 kJ per kg^{0.75} (SD: 193) per day for older cows.

Dry matter intake and basal ration intake were lowly positively correlated with milk yield (Table 4).

No correlations with milk yield were found for other variables of feeding behaviour (feeding rate, feeding and meal duration, and number of visits and meals). A low negative correlation was found between daily lying time and milk yield. Steps and number of lying bouts were not correlated with milk yield.

Low positive correlations were found between energy balance and number of meals, and between energy balance and number of visits. Feeding rate and total feeding time were not correlated with energy balance, but were highly negatively correlated with each other ($r: -0.71$; $P < 0.01$).

Low positive correlations were found between daily lying time and energy balance, dry matter intake ($r: 0.26$ $P: 0.02$), and basal ration intake ($r: 0.32$, $P < 0.01$). There were no correlations, however, between total feeding time or total meal time and daily lying time. Number of steps had low positive correlations with energy balance, daily meal duration ($r: 0.38$; $P < 0.01$), and daily feeding duration ($r: 0.29$; $P: 0.01$); and a moderate correlation with the number of visits to the feeder ($r: 0.54$; $P < 0.01$).

4. DISCUSSION

Compared with cows with no DP, cows with a short DP spent a longer time feeding but had a reduced feed intake (kg and NE) before calving. Other experimental studies with different DP lengths also

reported lower feed intake (DM) before calving for cows with a conventional or short DP than for cows with no DP (Rastani et al., 2005; van Knegsel et al., 2014). In the current study, the DP was accompanied by a DP ration, as is common in commercial dairy farming (Rastani et al., 2005; Santschi et al., 2011; Steeneveld et al., 2013). It is unlikely, however, that the reduced feed intake was due to the change from the lactation ration to the DP ration, because this reduction was also observed for cows with a short DP without a ration change (Rastani et al., 2005). This lower feed intake of dry cows likely reflects the lower energy requirement of dry cows. The lower feeding rate during the DP may be related to the high amount of fibre and the lower palatability of the DP ration (Baumont, 1996; Friggens et al., 1998).

Calving and the associated management had a large impact on feeding behaviour, irrespective of DP treatment. In the first week after calving, cows had more frequent, but shorter, meals, and increased feeding rates, overall resulting in a lower feed intake than in subsequent weeks. This suggests that the impact of calving and its associated management is not so much related to DP-related ration or group changes, and that the periparturient period will remain a period with quite extreme behavioural changes. Cows had about 7 meals per day before calving, and 8 meals per day after calving, irrespective of DP treatment. The use of separate meal criteria for feeding behaviour before calving and after calving did not influence this difference: conclusions were similar when a single meal criterion was used for both periods.

After calving, feed intake remained lower for cows with a short DP than for cows with no DP, despite being fed the same diet. Rastani et al. (2005) also reported a lower feed intake for cows with a short DP than for cows with no DP in the first 3 weeks after calving, whereas Van Knegsel et al. (2014) did not find a difference in feed intake between cows with a short DP and cows with no DP in the first 14 weeks after calving. It is unclear why cows with a short DP had a lower feeding rate than cows with no DP after calving. This might be related to rumen adaptation after a change in diet (Martens et al., 2012), the onset of lactation, or the change of social environment after calving. Further studies are needed in order to disentangle the impact of these factors. Possibly, cows with a short DP experience more inappetence or discomfort due to a more severe negative energy balance (Roche et al., 2009).

363 The higher energy intake and lower milk yield of cows with no DP compared with cows with a short
 364 DP may reduce the risk of metabolic diseases and improve welfare in early lactation (Ingvarsen,
 365 2006).

366 Cows with a short DP on average had a lower step count before calving than cows with no DP. This
 367 could be a direct consequence of the absence of the milking procedure, because going through the
 368 milking parlour (to be weighed) increased the step count of cows with a short DP by 220 steps.
 369 Excluding the day of weighing, cows with a short DP performed on average 624 steps per day, and
 370 cows with no DP performed 1117 steps per day. Twice-daily milking, therefore, could explain 89% of
 371 the difference $((2 \times 220)/(1117 - 624))$ in step count between cows with a short DP and cows with no
 372 DP, which suggests that the difference in steps was due to a difference in walking distance. Stepping
 373 could also occur without walking, e.g. as a restless behaviour in the milking parlour (Gygax et al.,
 374 2008). However, with stepping rates of less than 1 step per minute during the preparation and milking
 375 phases (Gygax et al., 2008), this is unlikely to contribute much to the observed difference in steps per
 376 day. Pen size was smaller for dry cows than for lactating cows, because the density was maintained at
 377 7 m² per cow and the dry cow group mostly consisted of 6 or fewer cows at a time. This could have
 378 further reduced the number of steps of cows with a short DP. Previous research showed that lactating
 379 cows walked more in larger pens (Telezhenko et al., 2012).

380 It could be questioned whether the reduced number of steps during the DP is beneficial, or whether
 381 the reduced physical activity might be a risk factor for cow health. Walking distance of housed cows
 382 is already limited compared with walking distance of grazing cows. For example, studies reported
 383 walking distances of 233 m per day for housed cows versus 2170 m for cows on pasture (Olmos et al.,
 384 2009), and step counts of 1506 versus 4064 steps per day (Dohme-Meier et al., 2014). Studies showed
 385 that exercise is beneficial for health in early lactation and for fitness of lactating and dry dairy cows
 386 (Gustafson, 1993; Davidson and Beede, 2009). In humans, women who continued to exercise
 387 regularly throughout pregnancy had a lower incidence of operative delivery, and had shorter active
 388 labour than women who discontinued their exercise (Clapp, 1990).

Before calving, the daily lying time of cows with no DP (12.6 h) was lower than for cows with a short DP (13.7 h), but higher than previously reported lying times of dry cows of 11.7 and 12.2 h per day (Huzzey et al., 2005; Schirmann et al., 2011). These lying times likely reflect the overall response to the environment in late gestation, as opposed to a short-term response to changes in management, because cows were regrouped and rations were switched 11 days before the measurement period, and cows were dried off 4 days before the measurement period. Cows in both DP treatments spent less time lying after calving than before calving. Other studies found that lying time was lower in early lactation than later in lactation (Munksgaard et al., 2005; Bewley et al., 2010). Due to the short lying time, hormonal changes, and a negative energy balance, cows in early lactation are particularly susceptible to lameness (Cook and Nordlund, 2009). The no DP treatment increased daily lying time after calving by 0.9 h compared with a short DP, which might reduce the risk of developing lameness in early lactation.

Higher daily lying time was lowly associated with lower milk yield in early lactation. It has been suggested that cows with higher yields and a more severe negative energy balance have to spend more time feeding, and can consequently spend less time lying (Roche et al., 2009; Bewley et al., 2010). In the current study, however, cows with a short DP and cows with no DP spent less time feeding and less time lying after calving than before calving. For cows with a short DP, the reduction in feeding and lying time may be related to the twice-daily milking after calving, compared with no milking before calving, that reduced their time budget for other behaviours. This was not the case for cows with no DP, however, because they were milked both before and after calving. Moreover, daily feeding time was not associated with daily lying time, or with milk yield. Therefore, lying time was probably not constrained by feeding time. Norring et al. (2012) also found a negative association between daily lying time and milk yield at 8 weeks in milk, with no associations between milk yield and feeding time. Løvendahl and Munksgaard (2016) found a positive correlation between milk yield and feeding time and a negative correlation between milk yield and lying time in primiparous cows. Other factors may explain why a higher milk yield was associated with a shorter lying time in early lactation. Considering that level of milk yield relates to udder pressure (Bertulat et al., 2013), cows

with higher milk yields may experience discomfort when lying down and therefore lie down less. There was a low positive association between energy balance and daily lying time in this study. A prolonged negative energy balance might also cause discomfort due to hunger, weariness, or (subclinical) metabolic disorders, which might reduce lying behaviour (Roche et al., 2009). Not subjecting cows to a DP could improve cow welfare. It does not require cessation of lactation, or ration and group changes commonly associated with a DP. During late gestation, cows with no DP spent more than 12 h per day lying, without the reduction in steps that was seen in cows with a short DP. In early lactation, cows with no DP had a higher feed intake, improved energy balance, and increased lying time compared with cows with a short DP. In the current study, the impact of being dry cannot be separated from the impact of group and ration changes. The impact of DP management might be lessened through technical solutions. For example, separation gates can divert dry cows away from the milking parlour and towards a DP ration, and thereby facilitate that dry cows remain in the lactating herd. Moreover, a short DP can be applied without a change in ration (Rastani et al., 2005). An experimental design in which dry and lactating cows remain in the same herd is necessary to research the impact of being dry as such.

5. CONCLUSION

Cows with a short DP appeared to get more rest than cows with no DP: they had lower step counts and longer lying and feeding times in late gestation. The differences in number of steps and feed intake seemed direct consequences of not being milked. Cows with no DP also had longer lying times (exceeding 12 h per day) before calving than in early lactation, despite the twice-daily milking. After calving, cows with no DP had longer lying times and ate more than cows with a short DP. Not subjecting cows to a DP may improve cow welfare through absence of DP-related changes in management (i.e. cessation of lactation, ration and group changes), increased walking activity in late gestation, and a better feed intake and longer lying time in early lactation.

ACKNOWLEDGEMENT

The authors thank the staff of the Dairy Campus and P. van der Spek for support during the experiment, and C. G. van Reenen and J. T. N. van der Werf for sharing the IceManager software.

443 This study is financed by DairyNL (Zuivel NL; organization of the Dutch dairy supply chain) and the
444 Dutch Ministry of Economic Affairs (EZ), as part of the Sustainable Dairy Chain initiative (Duurzame
445 Zuivelketen). The authors gratefully acknowledge DairyCare (COST Action FA1308) for funding a
446 Short Term Scientific Mission for the first author at SRUC.

REFERENCES

- Baumont, R. 1996. Palatability and feeding behaviour in ruminants. A review. *Ann. Zootech.* 45:385–400. doi:10.1051/animres:19960501.
- Bertulat, S., C. Fischer-Tenhagen, V. Suthar, E. Möstl, N. Isaka, and W. Heuwieser. 2013. Measurement of fecal glucocorticoid metabolites and evaluation of udder characteristics to estimate stress after sudden dry-off in dairy cows with different milk yields. *J. Dairy Sci.* 96:3774–87. doi:10.3168/jds.2012-6425.
- Bewley, J.M., R.E. Boyce, J. Hockin, L. Munksgaard, S.D. Eicher, M.E. Einstein, and M.M. Schutz. 2010. Influence of milk yield, stage of lactation, and body condition on dairy cattle lying behaviour measured using an automated activity monitoring sensor. *J. Dairy Res.* 77:1–6. doi:10.1017/S0022029909990227.
- Butler, W.R. 2003. Energy balance relationships with follicular development, ovulation and fertility in postpartum dairy cows. *Livest. Prod. Sci.* 83:211–218.
- Capuco, A. V, R.M. Akers, and J.J. Smith. 1997. Mammary growth in Holstein cows during the dry period: quantification of nucleic acids and histology. *J. Dairy Sci.* 80:477–87. doi:10.3168/jds.S0022-0302(97)75960-5.
- Chen, J., J.J. Gross, H.A. van Dorland, G.J. Remmelink, R.M. Bruckmaier, B. Kemp, and A.T.M. van Knegsel. 2015a. Effects of dry period length and dietary energy source on metabolic status and hepatic gene expression of dairy cows in early lactation. *J. Dairy Sci.* 98:1033–1045. doi:10.3168/jds.2014-8612.
- Chen, J., N.M. Soede, H.A. van Dorland, G.J. Remmelink, R.M. Bruckmaier, B. Kemp, and A.T.M. van Knegsel. 2015b. Relationship between metabolism and ovarian activity in dairy cows with different dry period lengths. *Theriogenology*. 84:1387–1396. doi:10.1016/j.theriogenology.2015.07.025.
- Clapp, J.F. 1990. The course of labor after endurance exercise during pregnancy. *Am. J. Obstet. Gynecol.* 163:1799–1805. doi:10.1016/0002-9378(90)90753-T.
- Cook, N.B., and K. V Nordlund. 2009. The influence of the environment on dairy cow behavior, claw health and herd lameness dynamics. *Vet. J.* 179:360–9. doi:10.1016/j.tvjl.2007.09.016.
- Davidson, J.A., and D.K. Beede. 2009. Exercise training of late-pregnant and nonpregnant dairy cows affects physical fitness and acid-base homeostasis. *J. Dairy Sci.* 92:548–62. doi:10.3168/jds.2008-1458.
- Dohme-Meier, F., L.D. Kaufmann, S. Görs, P. Junghans, C.C. Metges, H.A. Van Dorland, R.M. Bruckmaier, and A. Mürner. 2014. Comparison of energy expenditure, eating pattern and physical activity of grazing and zero-grazing dairy cows at different time points during lactation. *Livest. Sci.* 162:86–96. doi:10.1016/j.livsci.2014.01.006.
- Fraser, D., D.M. Weary, E.A. Pajor, and B.N. Milligan. 1997. A scientific conception of animal welfare that reflects ethical concerns. *Anim. Welf.* 6:187–205.
- Fregonesi, J.A., and J.D. Leaver. 2001. Behaviour, performance and health indicators of welfare for dairy cows housed in strawyard or cubicle systems. *Livest. Prod. Sci.* 68:205–216. doi:10.1016/S0301-6226(00)00234-7.
- Fregonesi, J.A., C.B. Tucker, and D.M. Weary. 2007. Overstocking reduces lying time in dairy cows. *J. Dairy Sci.* 90:3349–54. doi:10.3168/jds.2006-794.
- Friggens, N.C., B.L. Nielsen, I. Kyriazakis, B.J. Tolcamp, and G.C. Emmans. 1998. Effects of feed composition and stage of lactation on the short-term feeding behavior of dairy cows. *J. Dairy Sci.* 81:3268–3277. doi:10.3168/jds.S0022-0302(98)75891-6.
- Gomez, A., and N.B. Cook. 2010. Time budgets of lactating dairy cattle in commercial freestall herds.

493 *J. Dairy Sci.* 93:5772–81. doi:10.3168/jds.2010-3436.

494 González, L.A., B.J. Tolkamp, M.P. Coffey, A. Ferret, and I. Kyriazakis. 2008. Changes in feeding
 495 behavior as possible indicators for the automatic monitoring of health disorders in dairy cows. *J.*
 496 *Dairy Sci.* 91:1017–28. doi:10.3168/jds.2007-0530.

497 Gustafson, G.M. 1993. Effects of daily exercise on the health of tied dairy cows. *Prev. Vet. Med.*
 498 17:209–223. doi:10.1016/0167-5877(93)90030-W.

499 Gygas, L., I. Neuffer, C. Kaufmann, R. Hauser, and B. Wechsler. 2008. Restlessness behaviour, heart
 500 rate and heart-rate variability of dairy cows milked in two types of automatic milking systems
 501 and auto-tandem milking parlours. *Appl. Anim. Behav. Sci.* 109:167–179.
 502 doi:10.1016/j.applanim.2007.03.010.

503 Huzzey, J.M., M.A.G. von Keyserlingk, and D.M. Weary. 2005. Changes in feeding, drinking, and
 504 standing behavior of dairy cows during the transition period. *J. Dairy Sci.* 88:2454–61.
 505 doi:10.3168/jds.S0022-0302(05)72923-4.

506 Huzzey, J.M., T.J. de Vries, P. Valois, and M.A.G. von Keyserlingk. 2006. Stocking density and feed
 507 barrier design affect the feeding and social behavior of dairy cattle. *J. Dairy Sci.* 89:126–33.
 508 doi:10.3168/jds.S0022-0302(06)72075-6.

509 Ingvarsen, K.L. 2006. Feeding- and management-related diseases in the transition cow; Physiological
 510 adaptations around calving and strategies to reduce feeding-related diseases. *Anim. Feed Sci.*
 511 *Technol.* 126:175–213. doi:10.1016/j.anifeedsci.2005.08.003.

512 Kenward, M.G., and J.H. Roger. 1997. Small Sample Inference for Fixed Effects from Restricted
 513 Maximum Likelihood. *Biometrics.* 53:983–997.

514 von Keyserlingk, M.A.G., D. Olenick, and D.M. Weary. 2008. Acute behavioral effects of regrouping
 515 dairy cows. *J. Dairy Sci.* 91:1011–1016. doi:10.3168/jds.2007-0532.

516 van Kneysel, A.T.M., S.G.A. van der Drift, J. Cermáková, and B. Kemp. 2013. Effects of shortening
 517 the dry period of dairy cows on milk production, energy balance, health, and fertility: A
 518 systematic review. *Vet. J.* 198:707–13. doi:10.1016/j.tvjl.2013.10.005.

519 van Kneysel, A.T.M., G.J. Remmelink, S. Jorjeng, V. Fievez, and B. Kemp. 2014. Effect of dry
 520 period length and dietary energy source on energy balance, milk yield, and milk composition of
 521 dairy cows. *J. Dairy Sci.* 97:1499–1512. doi:10.3168/jds.2013-7391.

522 Kok, A., A.T.M. van Kneysel, C.E. van Middelaar, H. Hogeveen, B. Kemp, and I.J.M. de Boer. 2015.
 523 Technical note: Validation of sensor-recorded lying bouts in lactating dairy cows using a 2-
 524 sensor approach. *J. Dairy Sci.* 98:7911–7916. doi:10.3168/jds.2015-9554.

525 Korte, S.M., B. Olivier, and J.M. Koolhaas. 2007. A new animal welfare concept based on allostasis.
 526 *Physiol. Behav.* 92:422–428. doi:10.1016/j.physbeh.2006.10.018.

527 Krohn, C.C., L. Munksgaard, and B. Jonassen. 1992. Behaviour of dairy cows kept in extensive (loose
 528 housing / pasture) or intensive (tie stall) environments - I. Experimental procedure, facilities,
 529 time budgets - diurnal and seasonal conditions. *Appl. Anim. Behav. Sci.* 34:37–47.

530 Kuhn, M.T., J.L. Hutchison, and H.D. Norman. 2005. Minimum days dry to maximize milk yield in
 531 subsequent lactation. *Anim. Res.* 54:351–367. doi:10.1051/animres.

532 Løvendahl, P., and L. Munksgaard. 2016. An investigation into genetic and phenotypic variation in
 533 time budgets and yield of dairy cows. *J. Dairy Sci.* 99:408–17. doi:10.3168/jds.2015-9838.

534 Martens, H., I. Rabbani, Z. Shen, F. Stumpff, and C. Deiner. 2012. Changes in rumen absorption
 535 processes during transition. *Anim. Feed Sci. Technol.* 172:95–102.
 536 doi:10.1016/j.anifeedsci.2011.12.011.

537 Martin, P., and P. Bateson. 1993. Measuring behaviour. 2nd ed. Cambridge University Press,

538 Cambridge, UK. 222 pp.

539 Munksgaard, L., M.B. Jensen, L.J. Pedersen, S.W. Hansen, and L. Matthews. 2005. Quantifying
540 behavioural priorities—effects of time constraints on behaviour of dairy cows, *Bos taurus*. *Appl.*
541 *Anim. Behav. Sci.* 92:3–14. doi:10.1016/j.applanim.2004.11.005.

542 Norring, M., A. Valros, and L. Munksgaard. 2012. Milk yield affects time budget of dairy cows in tie-
543 stalls. *J. Dairy Sci.* 95:102–8. doi:10.3168/jds.2010-3458.

544 Olmos, G., L. Boyle, A. Hanlon, J. Patton, J.J. Murphy, and J.F. Mee. 2009. Hoof disorders,
545 locomotion ability and lying times of cubicle-housed compared to pasture-based dairy cows.
546 *Livest. Sci.* 125:199–207. doi:10.1016/j.livsci.2009.04.009.

547 Pezeshki, A., J. Mehrzad, G.R. Ghorbani, H.R. Rahmani, R.J. Collier, and C. Burvenich. 2007.
548 Effects of short dry periods on performance and metabolic status in Holstein dairy cows. *J.*
549 *Dairy Sci.* 90:5531–5541. doi:10.3168/jds.2007-0359.

550 Rajala-Schultz, P.J., J.S. Hogan, and K.L. Smith. 2005. Short communication: association between
551 milk yield at dry-off and probability of intramammary infections at calving. *J. Dairy Sci.*
552 88:577–9. doi:10.3168/jds.S0022-0302(05)72720-X.

553 Rastani, R.R., R.R. Grummer, S.J. Bertics, A. Gümen, M.C. Wiltbank, D.G. Mashek, and M.C.
554 Schwab. 2005. Reducing dry period length to simplify feeding transition cows: milk production,
555 energy balance, and metabolic profiles. *J. Dairy Sci.* 88:1004–14. doi:10.3168/jds.S0022-
556 0302(05)72768-5.

557 Robert, A., H. Seegers, and N. Bareille. 2006. Incidence of intramammary infections during the dry
558 period without or with antibiotic treatment in dairy cows - a quantitative analysis of published
559 data. *Vet. Res.* 37:25–48. doi:10.1051/vetres:2005047 25.

560 Roche, J.R., N.C. Friggens, J.K. Kay, M.W. Fisher, K.J. Stafford, and D.P. Berry. 2009. Invited
561 review: Body condition score and its association with dairy cow productivity, health, and
562 welfare. *J. Dairy Sci.* 92:5769–801. doi:10.3168/jds.2009-2431.

563 Santschi, D.E., and D.M. Lefebvre. 2014. Review : Practical concepts on short dry period
564 management. *Can. J. Anim. Sci.* 4:1–10. doi:10.4141/CJAS2013-194.

565 Santschi, D.E., D.M. Lefebvre, R.I. Cue, C.L. Girard, and D. Pellerin. 2011. Complete-lactation milk
566 and component yields following a short (35-d) or a conventional (60-d) dry period management
567 strategy in commercial Holstein herds. *J. Dairy Sci.* 94:2302–11. doi:10.3168/jds.2010-3594.

568 Schirmann, K., N. Chapinal, D.M. Weary, W. Heuwieser, and M. a G. von Keyserlingk. 2011. Short-
569 term effects of regrouping on behavior of prepartum dairy cows. *J. Dairy Sci.* 94:2312–2319.
570 doi:10.3168/jds.2010-3639.

571 Steeneveld, W., Y.H. Schukken, A.T.M. van Knegsel, and H. Hogeveen. 2013. Effect of different dry
572 period lengths on milk production and somatic cell count in subsequent lactations in commercial
573 Dutch dairy herds. *J. Dairy Sci.* 96:2988–3001. doi:10.3168/jds.2012-6297.

574 Telezhenko, E., M.A.G. von Keyserlingk, A. Talebi, and D.M. Weary. 2012. Effect of pen size, group
575 size, and stocking density on activity in freestall-housed dairy cows. *J. Dairy Sci.* 95:3064–9.
576 doi:10.3168/jds.2011-4953.

577 Tolkamp, B., D. Allcroft, J. Barrio, T. Bley, J. Howie, T. Jacobsen, C. Morgan, D. Schweitzer, S.
578 Wilnson, M. Yeates, and I. Kyriazakis. 2011. The temporal structure of feeding behavior. *Am.*
579 *J. Physiol. Integr. Comp. Physiol.* 301:378–393. doi:10.1152/ajpregu.00661.2010.

580 Tolkamp, B.J., N.C. Friggens, G.C. Emmans, I. Kyriazakis, and J.D. Oldham. 2002. Meal patterns of
581 dairy cows consuming mixed foods with a high or a low ratio of concentrate to grass silage.
582 *Anim. Sci.* 74:369–382.

583 Tucker, C.B., S.J. Lacy-Hulbert, and J.R. Webster. 2009. Effect of milking frequency and feeding

584 level before and after dry off on dairy cattle behavior and udder characteristics. *J. Dairy Sci.*
585 92:3194–3203. doi:10.3168/jds.2008-1930.

586 Winter, A., and J.E. Hillerton. 1995. Behaviour associated with feeding and milking of early lactation
587 cows housed in an experimental automatic milking system. *Appl. Anim. Behav. Sci.* 46:1–15.
588 doi:10.1016/0168-1591(95)00628-1.

589 Yeates, M.P., B.J. Tolkamp, D.J. Allcroft, and I. Kyriazakis. 2001. The use of Mixed Distribution
590 Models to Determine Bout Criteria for Analysis of Animal Behaviour. *J. Theor. Biol.* 213:413–
591 425. doi:10.1006/jtbi.2001.2425.

592 Zobel, G., D.M. Weary, K.E. Leslie, and M.A.G. von Keyserlingk. 2015. Invited review: Cessation of
593 lactation: Effects on animal welfare. *J. Dairy Sci.* 98:8263–8277. doi:10.3168/jds.2015-9617.

594 Under review:

595 van Hoeij, R.J., J. Dijkstra, R.M. Bruckmaier, J.J. Gross, T.J.G.M. Lam, G.J. Remmelink, B. Kemp,
596 A.T.M. van Kneegsel. The effect of dry period length and postpartum level of concentrate on
597 energy balance and plasma metabolites of dairy cows across the dry period and in early
598 lactation, under review

599